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(57) Abstract

A method for diagnosing psychiatric disorders, or gauging the effect of a treatment upon a psychiatric patient comprising: (a) determining the function and/or the level of at least one receptor-coupled G-protein; (b) diagnosing the psychiatric disorder, or gauging the effect of the treatment upon the patient, based on said determination in (a).

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METHODS AND KITS FOR DIAGNOSIS AND MONITORING TREATMENTS OF PSYCHIATRIC DISORDERS

FIELD OF THE INVENTION

The present invention relates to differential biochemical diagnosis of psychiatric disorders and, more particularly, to the use of the determination of the function and level of certain proteins in the accurate diagnosis of a variety of psychiatric disorders and for accurately following the progress of treatment of such disorders.

BACKGROUND OF THE INVENTION

Presently used differential diagnosis techniques for the classification of psychiatric disorders are largely phenomenological. Diagnosis is based on the observation of certain symptoms and the course of the disorder. The presently used Diagnostic and Statistical Manual of Mental Disorders (DSM IIIR) is empirically-oriented and makes use of operational criteria which are predominantly phenomenological.

Considerable research effort has been directed at attempting to decipher the biological underpinnings of psychiatric disorders. Various hypotheses have been put forward as to the involvement of a variety of neurotransmitters and their receptors in the pathogenesis of psychiatric disorders and the mechanisms of various biological treatments used to combat these disorders. Yet, no biological or biochemical routine laboratory cross diagnostic tests which are capable of supporting psychiatric diagnosis are available. The need for laboratory tests of a biochemical nature, which will help in differential diagnosis in psychiatry is enormous and well recognized.

There is a widely recognized need for, and it would be highly advantageous to have, a scientifically-established biochemical assay for the differential diagnosis of psychiatric disorders. Such an assay would help in the differential diagnosis of hospitalized patients, outpatients, and patients treated by general practitioners. Of special importance is the differential diagnosis following the first psychotic episode between the two major psychoses, schizophrenia and manic-depressive illness.

For example, using presently known phenomenological diagnosis techniques there are no pathognomonic signs which unambiguously diagnose for schizophrenia. Hence, a follow-up diagnosis taking place at least six months following the initial diagnosis is required in order to reach a conclusive determination of schizophrenia.

Differentiating between the major psychoses has important prognostic and treatment implications and also has a number of highly desirable social outcomes. An important aspect of a biochemical assay for mental disorders such as major depression, is that it makes it possible for the general practitioner, who treats about half of major depression patients, and for mental health professionals, to decide on the desirability of pharmacological antidepressant treatments.

Another disorder which is normally first treated by general practitioners, and which usually requires a series of physical examinations and a battery of expensive laboratory studies to exclude a physical disorder, is panic disorder. An established biochemical assay for this disorder will aid in the early treatment of these patients using presently available, and effective, pharmacological treatments.

Various specific treatments are applied against mood disorders. Mood stabilizers like lithium and electroconvulsive treatment (ECT) are effective for the treatment of mania and depression, and for the prevention of both affective states. Antidepressant drugs are used in the treatment and prevention of depression. All these treatments do not exert their therapeutic effects immediately, but within three weeks or one month. About 30% of the patients do not respond to a certain treatment, but may respond to another kind of treatment. It is therefore of prime importance also to have a biochemical test that enables to biochemically assess the responsiveness of a patient to a psychiatric treatment.

If the altered biochemical parameters determined in psychiatric patients by the diagnostic assay are indicators of the affective state of the illness, and are normalized following an efficient psychiatric treatment, then such an assay may also aid the practitioner to biochemically follow and gauge the effectiveness of these treatments.

There are indications in the prior art for possible involvement of signal transduction, and particularly for the involvement of members of the family of receptor-coupled G-proteins, in psychiatric disorders. For example, a reduction in the density of ß-adrenergic receptors in leukocyte membrane preparations has been shown in patients with depression, and a decreased ß-adrenergic receptor responsiveness has been shown in leukocytes of patients suffering from depression, through measurements of ß-adrenergic receptor-stimulated cAMP production. The degree of these changes in ß-adrenergic receptor density and responsiveness were found to be correlated with the severity of depression (for discussion see Mazzola-Pomietto et al., 1994)

The earliest recognized event in signal transduction beyond ß-adrenergic as well as other receptors, involves coupling of the activated receptor with a G-protein.

The family of G-proteins currently includes 12-15 already known individual proteins. G-proteins are composed of three subunits: α, β, and γ. The α- subunit contains the binding site for guanine nucleotides, and possesses GTPase activity. The α-subunit also contains the site for nicotinamide adenine nucleotide (NAD) - dependent ADP-ribosylation catalyzed by bacterial toxins. The heterogeneity of the α-subunit serves to divide G-proteins into the major classes ($G_{S,}$, G_i, G_q).

Receptors for stimulatory hormones, i.e., β-adrenergic receptors, interact with G_S, the G-protein which activates adenylate cyclase, while those of inhibitory ligands, e.g., M_2-muscarinic receptors, interact with adenylate cyclase inhibitory regulator G_i. G_q may be involved in coupling receptor activation to the breakdown of phosphatidylino-sitol -4,5-biphosphate by phospholipase C.

Stimulation by an agonist increases the binding of guanine nucleotide to G-proteins and leads to their activation. Based on this characteristic, guanine nucleotide binding assay, is used as an established test for G-protein function.

The function of receptor-coupled G-proteins was found to be differentially attenuated by lithium (Avissar et al., 1988), other antibipolar treatments, and antidepressant drugs (Avissar and Schreiber 1992a,b). Moreover, hyperactivity of ß-adrenergic coupled and muscarinic-coupled G-proteins was detected in MNL of patients with mania (Schreiber et al., 1991), and an elevated level of α subunit of G_s-protein ($G\alpha_s$) was found in postmortem cerebral cortices of bipolar patients (Young et al., 1991).

None of these publications address the possibility of using these findings for diagnosis of a psychiatric disorder, and certainly, even if taken together, there is no indication for the possibility of differential diagnosis of the major psychiatric disorders.

In the present invention measurements of receptor-coupled G-proteins function and level are used for differential diagnosis of a patient with a psychiatric disorder, and also for evaluation of the responsiveness of patients to psychiatric treatments.

SUMMARY OF THE INVENTION

According to the present invention there is provided a method for diagnosing psychiatric disorders or gauging the effect of a treatment on a psychiatric patient comprising: (a) determining the function and /or the level of at least one receptor-coupled G-protein; (b) diagnosing the psychiatric disorder, or gauging the effect of the treatment upon the patient based on the determination in (a).

According to a preferred embodiment of the present invention the function of receptor-coupled G-protein is measured as agonist-induced increase in guanine nucleotide binding capacity in patient's mononuclear leukocytes (MNL).

According to another preferred embodiment of the invention the level of receptor-coupled G-protein is quantified using antibodies against $G\alpha_s$ or $G\alpha_i$ subunits.

The present invention also provides kits for carrying out the method of the invention.

Other embodiments and aspects of the present invention are set forth in, or arise from the following description of the invention.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 A graphic representation depicting non-specific, basal and agonist-induced specific binding of ^{3}H-Gpp(NH)p to MNL obtained from a healthy volunteer, as described in Example 1.

Figs. 2(a-e) Representative Scatchard plots depicting the results showing basal and agonist-induced ^{3}H-Gpp(NH)p binding in MNL membranes from a healthy volunteer, and from 4 patients with different psychiatric disorders, as described in Examples 1 and 2.

Figs. 3(a-d) Scatter plots depicting the results showing altered agonist- induced increase in ^{3}H-Gpp(NH)p binding capacity in MNL membranes from patients with various psychiatric disorders compared to those of healthy volunteers, as described in Examples 1 and 2.

Fig. 4 Bar graphs depicting the results showing the differential pattern of agonist- induced increase in Gpp(NH)p binding capacity in MNL membranes from healthy volunteers (A); healthy volunteers after physical exercise (B); manic patients (C); depressed patients (D); patients with panic disorder (E); and schizophrenic patients (F), as described in Examples 1 and 2.

Fig. 5 - A representative immunoblot with MNL protein samples from psychiatric patients (A,C) and a healthy volunteer (B), obtained with anti-sera against $G\alpha_s$ and $G\alpha_i$, as described in Example 3.

Figs. 6(a,b) Scatter plots depicting the results showing the $G\alpha_s$ (6a) and $G\alpha_i$ (6b) relative immunoreactivity level in MNL from healthy volunteers and

from patients with various psychiatric disorders, as described in Example 3.

Figs. 7(a,b) Graphic representations of the results illustrating the correlation between isoproterenol (7a) and carbamylcholine (7b)-induced increase in ^{3}H-Gpp(NH)p binding capacity and Beck score, in patients with depression and in healthy volunteers, as described in Example 4.

Fig. 8 - A graphic representation of the results illustrating the normalization of β-adrenergic receptor coupled G-protein (β-G_P) function in depressed patients treated with antidepressants, as described in Example 5.

Figs. 9(a,b) Graphic representations of the results illustrating time-course improvement of biochemical and psychiatric parameters in depressed patients treated by electroconvulsive treatment, as described in Example 5.

DETAILED DESCRIPTION OF THE INVENTION

The invention is based on the discovery of a differential pattern of receptor-coupled G-proteins function and immunoreactive level in MNL from patients with the major mental disorders:

Mania - increase in β-adrenergic and muscarinic receptor-coupled G-proteins (β-G_P and M-G_P) function, and increase in immunoreactive level of $G\alpha_S$ and $G\alpha_i$, as compared to normal (control) individuals.

Depression - decrease in β-G_P and M-G_P function, and decrease in immunoreactive levels of $G\alpha_S$ and $G\alpha_i$, as compared to normal (control) individuals

Schizophrenia - Increase in dopamine receptor-coupled G-protein (D-G_P) function, as compared to normal (control) individuals; and control-like levels of β-G_P and M-G_P function, and control-like immunoreactive level of $G\alpha_S$ and $G\alpha_i$.

Panic Disorder - Increase in β-G_P function and decrease in M-G_P function as compared to normal (control) individuals; and control-like immunoreactive level of $G\alpha_S$ and $G\alpha_i$.

The invention is further based on the discovery that altered receptor-coupled G-proteins function and level in MNL are biochemical indicators of the effective state of the illness, and that treated asymptomatic patients show normal values.

The present invention provides a cross diagnostic biochemical assay capable of differentiating between mental disorders, based on G-protein functional and/or quantitative measurements. The present invention also provides for biochemical assay for evaluating a patient's responsiveness to a psychiatric treatment.

By the above "function of G-protein" it is meant the agonist-induced activation of G-protein. According to a preferred embodiment of the present invention G-protein function is determined as agonist-induced increase in guanine nucleotide binding capacity.

Preferred agonists for carrying out the method of the present invention are isoproterenol, carbamylcholine and dopamine, which activate β-adrenergic, muscarinic and dopaminergic receptors respectively. However, other β-adrenergic, M_2-muscarinic and $D_{1/5}$-dopaminergic agonists stimulating receptors

of similar nature are suitable, and their use is within the scope of the present invention.

According to another preferred embodiment of the present invention the above "quantitative measures" or "immunoreactive level" of G-protein is determined as the immunoreactive level of its α-subunit.

According to a further preferred embodiment of the invention the psychiatric disorders that are diagnosed by the method of the invention are: mania, depression, panic disorder and schizophrenia.

According to a preferred method of the invention, receptor-coupled G-protein function and level are determined in MNL from a patient, however other types of cells may also be suitable for carrying out the method of the invention. For example other peripheral blood cells or elements (such as platelets), or skin cells may be suitable for diagnosis. However it should be noted that different cells may present different levels and types of receptors, and therefore survey must first be performed in an already diagnosed population of patients as well as in a population of healthy subjects, to assess the compatibility of each type of cells for these purposes, and to calibrate the method for when these different cells are used.

In the present invention the determination of the function of one, two or three receptor-coupled G-proteins may be suitably and effectively used for diagnosis of psychiatric disorders, or for gauging the effect of psychiatric treatments. Indeed, depression can be diagnosed based on hypofunction of β-G_P, and mania can be diagnosed based on hyperfunction of M-G_P, with high values of specificity and sensitivity. However, in most cases the function of more than one receptor-coupled G-protein should be determined to enable accurate diagnosis. These include β-G_P and M-G_P function, for mania, depression and panic disorder, and D-G_P function with either β-G_P or M-G_P function, or both, for diagnosing

schizophrenia. Therefore for differential diagnosis of a patient, it is preferred that the function of all three (β-GP, M-GP and D-GP) be determined. The specificity of the test for diagnosing a certain disorder can be controlled by the determination of the minimal requirements and the threshold values.

However, for monitoring the effect of a psychiatric treatment, the determination of the function of one receptor-coupled G-protein is usually sufficient, preferably the one whose function in the acute state of the illness deviates most from the normal level. The evaluation is based on comparison of values obtained before and during the treatment, and a shift toward a normal value indicates responsiveness to the treatment.

According to a preferred embodiment of the present invention, receptor-coupled G-protein function is determined by guanine nucleotide binding essay. However, any other assay which enables the determination of agonist-induced activation of G-protein may also be used to carry out the method of the invention.

According to further preferred embodiment of the invention, the guanine nucleotide used in the assay is Gpp(NH)p. Other guanine nucleotides or analogs may be suitable, and their use is within the scope of the present invention. These may include for example GTPγS (Wieland T. and Jakobs K.H., 1994), or GTP-azidoanilide (a photolable compound that binds irreversibly to the G-protein following exposure to U V light, Laugwitz K. L. et al.,1994).

According to still another embodiment of the present invention the guanine nucleotide used in the assay is labeled. The label of the guanine nucleotide may be any kind of label which can be detected by appropriate detection means, such as radiolable, fluorescent label, etc. However, instead of being labeled, the bound guanine nucleotide may be detected using a labeled probe, which is capable of specifically binding to the complex G-protein-guanine nucleotide, or which is

capable of specifically binding to the guanine nucleotide itself, with the limitation that the probe be contacted with the sample only after separation of unbound material. Any procedure which generates a detectable change, which is proportionaly to the binding of the guanine nucleotide to the G-protein, can be exploited to carry out the method of the invention.

The function of G-proteins, determined as agonist-induced increase in binding capacity, may be calculated from multi-point measures of binding as described in the General Procedures and in Example 1. However, the assay may be simplified using single-point measures, at single concen-tration of guanine nucleotide. For Gpp(NH)p, similar values were obtained based on multiple-point measures and single-point measures, at concentration of 5 mM.

Diagnosis of psychiatric disorders or following the effect of psychiatric treatment according to the present invention may also be based on the determination of the level of Gα-subunits. Determination of the immunoreactive level of $G\alpha_s$ and $G\alpha_i$ has been performed by immunoblot analysis of MNL membrane-proteins which were first separated on SDS-PAGE. However, it is preferred for routine assays to determine the immunoreactivity of Gα-subunits without prior separation of the sample. Quantitative immuno-assay of different types of Gα-subunits can be performed in crude preparation of membranes (see for example Lesch K.P., Manjii H.K., 1992), and their use is in the scope of the present invention.

Determination of the immunoreactive level of $G\alpha_s$ and $G\alpha_i$ for the method of the invention may also be performed by an ELISA test. A method based on competitive ELISA has been used to quantify various types of Gα-subunits in membranes from brain of rats (Lesch K.P., and Manjii H.K., 1992). However, other variations of ELISA tests or other immuno-assays can be employed and their use is within the scope of the present invention.

Many other variations in the determination of G-protein function are also possible, for carrying out the method of the invention. For example: guanine nucleotide-G-protein complex may be trapped by contacting the sample at the end of the binding reaction with an immobilized antibody, or with an antibody which is attached to magnetic particles. This enables the washing of the unbound material, and avoids the need for filtration of the sample. Immuno-separation of G-protein-guanine-nucleotide complex has been disclosed in a different method (Friedman E., et al., 1993). The bound guanine nucleotide may then be detected directly (if it is labeled) or indirectly (by a labeled probe which is capable of specifically binding G-protein-guanine-nucleotide complex).

In another aspect the invention is directed to kits for carrying out the method of the invention. Kits are based on the determination of G-protein function or on the determination of the immunoreactive level of $G\alpha_s$ and/or $G\alpha_i$. The kits may be used for diagnosis of psychiatric disorders, or for following the effect of psychiatric treatments.

An illustrative kit based on G-protein function determination according to the method of the invention comprises:

(a) agonists of one, two or three receptors, wherein said receptors are selected from the group comprising:

(I) ß-adrenergic receptor
(ii) muscarinic receptor
(iii) dopamine receptor

(b) labeled guanine nucleotide

(c) the manufacturer's instructions for using the kit

Criteria for diagnosis of each of the major psychiatric disorders may also be included in the manufacturer's instructions.

An illustrative kit based on $G\alpha_s$ and/or $G\alpha_i$ determination according to the method of the invention comprises:

(a) 1 or 2 monoclonal or polyclonal antibodies selected from the group comprising anti-$G\alpha_s$ and anti-$G\alpha_i$;

(b) detectable probe which is capable of specifically binding to the antibody of (a);

(c) standard samples;

(d) the manufacturer's instructions for using the kit.

Standard samples should be included to enable normalization of the results, and comparison to known mean-value of normal subjects. Detailed instructions for normalization should be included in the manufacturer's instructions along with the criteria for diagnosis.

The invention will be further illustrated by the following illustrative and non-limiting examples.

GENERAL PROCEDURES

Patients: Samples of Patients with Psychiatric Disorders and Control Group of Healthy Volunteers: All patients were diagnosed according to DSM-IIIR criteria by at least two senior psychiatrists. Inclusion criteria were normal results of physical examination, electrocardiogram, and laboratory tests for renal, hepatic, hematologic, and thyroid function. When indicated, patients and healthy volunteers were also evaluated through the use of Beck Inventory of Depression. Patients consented to a 60 ml blood donation for the experiment. No psychiatric treatment was given to the patients during one month prior to referral. In case

of a history of treatment with depot antipsychotics (three of the patients with schizophrenia), it was verified that no such medication was given for at least two months prior to referral.

The group of schizophrenic patients with positive symptoms consisted of 13 male and 10 female hospitalized subjects, average age 31.4 (19-59) years, diagnosed as suffering from schizophrenia of paranoid or disorganized types with a course classified as acute, subchronic with acute exacerbation, or chronic with acute exacerbation. This group of patients presented predominantly positive symptomatology, assessed by the Positive and Negative Syndrome Scale, PANSS (Total PANSS score=92.8+5.5; Positive Scale=26.7$\pm$1.5). Eight of the patients, never before treated by dopamine antagonists, were examined during their first psychotic episode and the diagnosis of schizophrenia was reached after at least one year of clinical follow-up.

The group of manic patients consisted of 13 male and 7 female hospitalized subjects, average age 34.8 (20-57) years, suffering from acute mania.

The group of untreated patients with major depression (unipolar and bipolar) consisted of 15 female and 13 male subjects, average age 42.7 (20-68) years: 18 were outpatients and 10 were hospitalized.

The group of outpatients with panic disorder, with or without agoraphobia, consisted of 6 male and 7 female subjects, average age 37.5 (23-55) years.

The healthy volunteer group consisted of 17 men and 13 women, average age 38.9 (20-77) years.

MNL Isolation: MNL were isolated from 60 ml heparinized fresh blood of adult donors, using Ficoll-Paque gradient according to Boyum (Boyum A., 1968). Cells

were homogenized in 25 mM Tris-HCl, pH 7.4, and 1 mM dithiotreitol (DTT). The homogenate was passed through two layers of cheesecloth to remove debris, and membranes were collected by further centrifugation at 18,000 g for 10 minutes. Membranes were then either freshly used for the functional binding measures or suspended in homogenization buffer containing 1mM ATP, 1mM EGTA, 2mM Mg^{2+} and 30% sucrose w/v, and frozen at -70°C until assayed by the quantitative measures. Aliquots were taken for protein concentration determination using Bradford's standard assay.

Guanine Nucleotide Binding Assay: Guanine nucleotide binding assay was performed according to Avissar S. et al., (Avissar S. et al., 1988). Guanosine β,γ, imido triphosphate [Gpp(NH)p], a nonhydrolyzable analog of GTP which has higher affinity for G-proteins, was used in guanine nucleotide binding assay.

Binding reaction was carried out at various concentrations of ^{3}H-Gpp(NH)p (0.05-5μM). Total binding was measured by adding aliquots of 50μg of membrane proteins to a series of tubes containing reaction buffer (25 mM Tris-HCl, pH 7.4, 1mM ATP, 1mM Mg^{2+}, 1mM EGTA, and 1mM DTT), with varying concentration of ^{3}H-Gpp(NH)p, to a final volume of 200μl. The tubes were incubated for 10 minutes at room temperature (18°C - 25°C) and the reaction was terminated with 5 ml of ice-cold buffer (10mM Tris-HCl, pH 7.4; 100mM NaCl). The samples were filtered through GF/C Whatman filters. The filters were washed twice with 3 ml of cold buffer, and their radioactivity were determined. Non-specific binding, at each ^{3}H-Gpp(NH)p concentration, was measured in parallel, in the presence of 100μM unlabeled GTPγS. The binding reactions were carried out in triplicates, and specific binding was calculated by subtracting the nonspecific binding from the total binding.

Agonist-induced Binding: Three agonists were used, the β-adrenergic agonist - isoproterenol, the muscarinic agonist - carbamylcholine. and the dopamenergic

agonist-dopamine. The effects of the agonists on ^{3}H-Gpp(NH)p binding were assessed by adding isoproterenol (25 μM), carbamylcholine (50 μM) or dopamine (50 μM) to the reaction mixture. These represent minimal concentration resulting in maximal effect of the agonist.

Antagonists Effect on Agonist-induced Binding: Antagonists effects on agonist-induced increase in ^{3}H-Gpp(NH)p binding capacity were assessed by adding them to the reaction mixture, to the final concentrations indicated for each one. 1 μM propranolol, β-adrenergic antagonist; 10 μM ADFX116 and 10 μM pirenzepine, M_2 and M_1 muscarinic antagonists; 1 μM SCH23390, and 1 μM sulpiride, $D_{1/5}$ and D_2 dopaminergic receptor antagonists, respectively.

The Effect of Cholera and Pertussis Toxins on Agonist-induced Binding: MNL membranes (3-4mg) were suspended in 1 ml buffer containing 25 mM Tris-HCl, pH 7.4, 10mM NAD, 1mM ATP, 10 mM thymidine, and 100 μM GTP. ADP ribosylation was carried out for 15 minutes at 30°C by adding cholera toxin (20 μg/ml) preactivated for 10 minutes at 37°C with 20 mM DTT or pertussis toxin (10 μg/ml) preactivated for 10 minutes at 30°C with 20 mM DTT. The reaction was stopped by adding 25 ml ice-cold 25mM Tris-HCl, pH 7.4, immediately followed by centrifugation at 18,000 g for 10 minutes. ^{3}H-Gpp(NH)p binding was then carried out as described above.

Immunoblot Analysis: Membranes were thawed and aliquots of 10 μg membranes were taken for protein separation on SDS- (10%) polyacrylamide gel electrophoresis. The proteins were transferred to nitrocellulose paper by electroblotting apparatus. Blots were washed in Tris-buffered saline (TBS) containing 3% Tween-20, and blocked by incubation for 1 hr with 5% BSA in TBS containing 0.1% Tween-20 (TTBS). After two washes in TTBS, blots were incubated overnight with each of the following antisera (NEN-DuPont) directed specifically against α_S (dilution 1:2,500), $\alpha_{i1,i2}$ (dilution 1:5,000), followed by

subsequent incubation with goat anti-rabbit IgG labeled with horseradish peroxidase. Immunoreactivity was detected with the Enhanced Chemiluminescence Western Blot Detection System (Amersham) followed by exposure to Kodak X-Omat film. Peak heights of immunoreactive bands were determined with an image analysis system. The optical density of the immunoreactive bands was normalized against 10 μg rat cortical membranes, run in each blot as a standard value. Linearity of the assay with respect to protein concentration, was found in the range of 2.5- 15 μg membrane protein from a healthy volunteer.

Statistical Analysis: Dunn's test was used for non-parametric multiple comparisons of receptor-coupled G-protein functions and immunoreactive quantities in patients MNL against a single control group with unequal sample sizes, and the value of the test statistic was compared with the table of critical values of Q' (Zar J.H., 1984).

Example 1: Receptors-coupled G-protein function in healthy volunteers.

Binding of ^{3}H-Gpp(NH)p to MNL membranes from healthy volunteers was determined at various ^{3}H-Gpp(NH)p concentrations in the absence of an agonist or in the presence of isoproterenol, carbamylcholine or dopamine. Specific binding (calculated by subtracting the non-specific binding from the total binding) reached equilibrium within 5 min. and remained constant for at least 30 min.

Representative saturation curves of specific ^{3}H-Gpp(NH)p binding to MNL membranes from a healthy volunteer [in the absence of an agonist (open circles), and in the presence of isoproterenol (open triangles), carbamylcholine (closed squares), and dopamine (closed circles)and the parallel of non-specific binding curve (closed triangles) are presented in

Fig. 1. All three agonist-induced increase in Gpp(NH)p binding capacity without substantially effecting the affinity of binding.

Fig. 2a depicts representative Scatchard plots of basal and agonist-induced binding curves in MNL membranes from a healthy volunteer. The ratio of specifically bound Gpp(NH)p to free Gpp(NH)p is plotted against specifically bound Gpp(NH)p. Bmax, the maximal binding capacity can be estimated from the intercept of the extrapolated linear regression line with the horizontal axis.

Average over all the population of healthy volunteer subjects examined:

Bmax - Bmax basal value in the absence of an agonist is 40.3±1.0 pmole/mg protein

β-Bmax - Bmax value in the presence of β-adrenergic agonist isoproterenol is 50.9±1.9 pmole/mg protein ($Q'=3.2$ $p<0.01$)

M-Bmax - Bmax value in the presence of the muscarinic agonist carbamylcholine is 52.5±1.6 pmole/mg protein ($Q'=3.57$ $p<0.01$)

D-Bmax - Bmax value in the presence of the dopamine is 54.9±2.0 pmole/mg protein ($Q'=4.34$, $p<0.01$).

The increase in Gpp(NH)p binding capacity induced by all three agonists is statistically, highly significant.

The Bmax values did not show significant change with age, (L. Barki-Harrington et al., 1996, in press) or after physical exercise.

Agonist-induced increase in Gpp(NH)p binding capacity is calculated as :

$$\frac{\text{(Bmax in the presence of an agonist}}{\text{Bmax-basal level}} - 1) \times 100$$

Scatter plot of isoproterenol (β), carbamylcholine (M), and dopamine (D) induced increase in Gpp(NH)p binding capacity for a group of healthy volunteers (closed circles) is shown in Fig. 3d.

The calculated means, and standard error of the means of agonists induced increase in Gpp(NH)p binding capacity (Fig. 4) were 26.4% ± 1.3 for isoproterenol(open column), 28.3%±1.7 for carbamylcholine (dashed column), and 26.8%±1.9 for dopamine (closed column).

Table I discloses the effect of β-adrenergic, muscarinic and dopaminergic antagonists, and the effect of cholera and pertussis toxins on agonist-induced increase in Gpp(NH)p binding. Cholera toxin is known to inhibit G_S protein through ribosylation of its α subunit. As depicted in Table 1 the increase in Gpp(NH)p binding capacity induced by dopamine and isoproterenol was abolished by cholera toxin, suggesting their effects on G_S protein function. Human MNL are known to possess surface β-adrenergic receptors, which activate adenylyl cyclase function via G_S protein. Dopamine-induced increase in guanine nucleotide binding was specifically blocked by SCH-23390, a selective D_1 and D_5 antagonist, and was not effected by sulpiride, a selective D_2 antagonist. The D_1 and D_5 receptor-selective agonist SKF-38393 1 μM induced concentration-dependent increases in Gpp(NH)p binding capacity, which was similar to the increases induced by dopamine (not shown). D_1 and D_5 are the only dopamine receptors currently identified to activate adenylyl cyclase via G_S. However, by now, there are indications to the expression of the D_3 and the D_5 receptors only in human peripheral blood lymphocytes. Thus, dopamine probably effects G_S protein function in MNL through a D_5 receptor.

As depicted in Table 1 the effect of carbamylcholine on Gpp(NH)p binding was inhibited by pertussis toxin pretreatment, unaffected by cholera toxin, and specifically blocked by the M_2 antagonist ADFX116, while unaffected by the M_1

antagonist pirenzepine. M_2 receptors are coupled to G_i-proteins. G_i-proteins are known to be inhibited by pertussis toxin, through ribosylation of their α subunit. Thus, these findings suggest that carbamylcholine effects are exerted through M_2 receptors, coupled to a G_i, which inhibits adenylyl cyclase.

TABLE I:

Agonist	Antagonist	% increase in ^{3}H-Gpp(NH)p binding capacity		
			Cholera Toxin	Pertussis toxin
Isoproterenol		25.1 ± 3.1	<5[a]	29.9 ± 3.7
Isoproterenol	Propranolol	<5[a]		
Carbamylcholine		29.8 ± 2.1	25 ± 2.6	<5[a]
Carbamylcholine	ADFX116	<5[a]		
Carbamylcholine	Pirenzepine	31.9 ± 3.5		
Dopamine		26.4 ± 2.5	<5[a]	19.5 ± 3.9
Dopamine	SCH23390	<5[a]		
Dopamine	Sulpiride	27.8 ± 2.9		
Dopamine	Propranolol	22.6 ± 2.3		

[a] No statistically significant increases in ^{3}H-Gpp(NH)p binding capacity were detected.

Example 2: Receptor-coupled G-protein function in psychiatric patients.

Receptor induced G-protein functions were also assayed in patients with various psychiatric disorders. Fig. 2 (a-e) depicts representative examples of Gpp(NH)p binding assay in individual patients with mania (b); depression (c); schizophrenia (d); and panic disorder (e), as compared with a healthy volunteer (a). Binding was assayed under basal conditions (open circles) or in the presence of agonists: isoproterenol (open triangles), carbamylcholine (closed squares), or dopamine (closed circles)-[in (a) and (d) only]. No significant change in basal binding capacity was found between the five individuals. However, each

individual patient presented a specific, differential pattern of agonist induced increase in binding capacity.

Fig. 3(a-d) and Fig. 4 summarize the results of agonist-induced increase in binding capacity in groups of patients with the various psychiatric disorders. Scatter plots for patients (open circles) with mania (3a); depression (3b); schizophrenia (3c); and panic disorder (3d) as compared to healthy volunteers (closed circles) are presented in Fig. 3.

The calculated means and standard error of the means for isoproterenol (open columns), carbamylcholine (dashed columns), and dopamine (closed columns) induced increase in Gpp(NH)p binding capacity, are presented in
Fig. 4 for manic patients (C); depressed patients (D); patients with panic disorder (E); schizophrenic patients (F), and for healthy volunteers at rest (A); or after physical exercise (B).

Each group of patients is characterized by a distinct pattern of receptor-coupled G-protein function. In comparison to control subjects, MNL of patients with mania were characterized by elevated β-adrenergic receptor-coupled G protein (β-G_P) function and elevated muscarinic receptor-coupled G protein (M-G_P) function; reduced function of both β-G_P and M-G_P were detected in MNL of depressed patients. MNL of patients with panic disorder were characterized by elevated β-G_P function and reduced M-G_P function. In contrast to the above mentioned groups of patients, no differences were detected in the function of β-G_P and M-G_P between patients with schizophrenia and control subjects. In these patients elevated dopaminergic receptor-coupled G protein (D-G_P) function was detected in comparison to normal subjects.

The pattern of agonist-induced increase in Gpp(NH)p binding capacity in neuroleptic naive schizophrenic patients (8 of 23 patients) was similar to that of the other schizophrenic patients.

The changes in G-protein functions in the patients are not merely due to motor hyper- or hypoactivity, as normal subjects after intensive physical exercise showed G-proteins functions no different from the control subjects.

The results are summarized in Table II.

TABLE II:

	Agonist	**Agonist-induced increase in Gpp(NH)p binding capacity**	**Significance of the difference (in comparison to control).**
Control Subject	Isoproterenol	26.4 ± 1.3	
	Carbamylcholine	28.3 ± 1.7	
	Dopamine	26.8 ± 1.9	
Mania	Isoproterenol	72.7 ± 6.5	Q' = 5.00 p<0.01
	Carbamylcholine	63.8 ± 7.9	Q' = 3.78 p<0.01
Depression	Isoproterenol	2.0 ± 1.2	Q' = 4.44 p>0.01
	Carbamylcholine	10.5 ± 2.9	Q' = 3.79 p<0.01
Panic	Isoproterenol	57.8 ± 5.9	Q' = 3.26 p<0.01
	Carbamylcholine	14.3 ± 3.2	Q' = 2.65 p<0.01
Schizophrenia	Isoproterenol	29.5 ± 1.9%	no significant differ.
	Carbamylcholine	30.8 ± 3.0%	no significant differ.
	Dopamine	76.5 ± 8.2%	Q' 5.56 p<0.01

Comparable results were obtained when specific binding (basal and agonist-induced) at 5mM was used as single point measure for calculating agonist-induced increase in Gpp(NH)p binding capacity.

The empirical findings may potentially be used for differential diagnosis of psychiatric patients.

The sensitivity and specificity of a potential diagnostic assay which is based on a G-protein function measurements can be estimated using validity test, for any predetermined threshold values, and for predetermined test criteria.

For example, by determining the values of $\geq$ 45% and $\leq$ 15% agonist-induced increase in Gpp(NH)p binding capacity as threshold values reflecting G-protein hyperfunction and hypofunction respectively, the following results were obtained:

For the diagnosis of mania requiring hyperfunction of both β-G_P and M-G_P the sensitivity was found to be 0.8 and the specificity 0.93.

For the diagnosis of schizophrenia, requiring hyperfunction of D-G_P and normal β-G_P and M-G_P, the sensitivity was found to be 0.87 and the specificity 0.97.

For the diagnosis of depression requiring hypofunctional of β-G_P and G_P, the sensitivity was found to be 0.89 and the specificity 0.90.

For the diagnosis of panic disorder requiring hyperfunction of β-G_P with normal or hypofunction of M-G_P, the sensitivity was found to be 0.75 and the specificity 0.93.

Analysis of the results may be based on values obtained with one (only in two cases), two or three agonists and different threshold may be used for each of them, according to the distribution of values found in patients and control subjects. Other manipulation of the assay results are also possible and may aid

in reliable diagnosis. For example, the sensitivity and specificity of the diagnosis of panic disorder may be elevated if evaluation is based on the ratio β-G_P/M-G_P, rather than on each parameter separately.

Example 3: $G\alpha_s$ and $G\alpha_i$ levels in psychiatric patients.

The level of G_s and G_i proteins in MNL was determined by immunoblot analysis using polyclonal antibodies against $G\alpha_s$ and $G\alpha_i$. A representative immunoblot with MNL-membrane proteins from a patient with mania (A); a patient with depression (C); and an age and sex matched healthy volunteer (B) is shown in Fig. 5. Of the two types of $G\alpha_s$ recognized by anti- $G\alpha_s$ (45kDa and 52 kDa), only the 45 kDa species $G\alpha_s$ was detected in MNL.

Normalized data are presented by scatter plots (Fig. 6). The level of the 45 kDa species of $G\alpha_s$ (6a) and the level of $G\alpha_i$ (6b) were significantly elevated in patients with mania (closed squares), and reduced in depressed patients (closed circles), in comparison with control subjects (open circles). No significant change was detected in MNL from patients with schizophrenia (closed triangles), or panic disorder (not shown).

Determining the value of $\geq$ 15% elevation or reduction in $G\alpha_s$ and $G\alpha_i$ immunoreactive level compared to the mean values of control group as a threshold, the specificity and selectivity of a test can be evaluated. For diagnosing of mania requiring elevation of $\geq$ 15% in the immunoreactive level of at least one type of Gα-subunit ($G\alpha_s$ or $G\alpha_i$), the sensitivity is 0.73 and the selectivity 0.81. For diagnosing of depression requiring reduction of $\geq$ 15% in the immunoreactive level of at least one type of Gα-subunit ($G\alpha_s$ or $G\alpha_i$), the sensitivity is 0.73 and the selectivity 0.90.

Example 4: Correlation between the biochemical parameters and psychiatric evaluation of depression.

Normal subjects, outpatients with mild depression and patients with moderate and severe depression, were evaluated through the use of Beck Inventory of Depression and their β-G_P function and M-G_P function, as well as $G\alpha_s$ and $G\alpha_i$ level were simultaneously analyzed.

As depicted in Fig. 7, isoproterenol (7a) and carbamylcholine (7b)-induced increase in Gpp(NH)p binding capacity were found to be correlated, in a statistically highly significant manner with the scoring of Beck Inventory (Spearman correlation efficient = −0.781 $p<0.001$ and −0.749 $p<0.001$ respectively). Significant correlations were also obtained between $G\alpha_s$ and $G\alpha_i$ immunoreactivity level and Beck Score (−0.63 $p<0.001$ and −0.693 $p<0.001$ respectively), (not shown).

Example 5: The effect of psychiatric treatments on the function and the level of G-proteins.

β-G_P and M-G_P function(s) in lithium treated euthymic bipolar patients were found to be similar to these of control subjects (Schreiber at al 1991, Avissar and Schreiber 1992a).

Fig. 8 depicts β-G_P function in depressive patients before (closed circles) and following treatments with antidepressants (closed circles). The alterations in β-G_P function in most (7of 8) of the depressive patients examined were normalized following treatments with antidepressants. The only exception being a patient who was also found to be non-responsive to the treatment based on psychiatric parameters.

β-G_P function (open circles) and M-G_P function (open squares), were measured in MNL membranes from depressed patients at intervals during ECT treatment.

The state of the psychiatric illness was evaluated in parallel, using Beck Inventory for Depression (closed circles) or Hamilton and Brief Psychiatric Rating Scale (BPRS) Scores (not shown).

Fig. 9a depicts representative examples of the dynamics of the change in β-G_P function (open circles), M-G_P function (open squares), and Beck score (closed circles) in 4 depressed patients, on electroconvulsive treatment [ECT].

Fig. 9b depicts representative examples of the dynamics of the change in $G\alpha_s$ (open circles), $G\alpha_i$ (open squares) immunoreactive level, and Beck score (closed circles) in 4 depressed patients, on electroconvulsive treatment [ECT].

From the above mentioned Figures it is apparent that the normalization of all 4 biochemical parameters precedes the clinical improvement. Similar results were obtained in another 10 patients who, according to psychiatric criteria, were responsive to the treatment. Patients who, by psychiatric criteria, did not respond to ETC treatment, did not show normalization of the hypofunction and the reduced immunoreactivity (not shown).

Thus G-protein functional and quantitative measurements may serve as predictors for the responsiveness of psychiatric patients to various psychiatric treatments..

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CLAIMS

1. A method for diagnosing psychiatric disorders, or gauging the effect of a treatment upon a psychiatric patient comprising:

   (a) determining the function and/or the level of at least one receptor - coupled G-protein;
   (b) diagnosing the psychiatric disorder, or gauging the effect of the treatment upon the patient, based on said determination in (a).

2. A method for diagnosing psychiatric disorders, or gauging the effect of a treatment upon a psychiatric patient comprising:

   (a) determining the function and/or the level of at least one receptor coupled G-protein in mononuclear leukocytes (MNL) isolated from the patient:
   (b) diagnosing the psychiatric disorder or gauging the effect of the treatment upon the patient based on said determination in (a).

3. A method according to claim 1 or claim 2 for diagnosing psychiatric disorders, or gauging the effect of a treatment upon a psychiatric patient comprising:

   (a) determining the function of one, two or three receptor-coupled G-proteins, wherein said G-proteins are selected from
      (i) ß-adrenergic receptor-coupled G-protein,
      (ii) muscarinic receptor coupled G-protein, and
      (iii) dopamine receptor-coupled G-protein;

   (b) diagnosing the psychiatric disorder or gauging the effect of the treatment upon the patient based on said determination in (a).

4. A method according to any one of claims 1 to 3 wherein the receptor-coupled G-protein function is determined by a guanine nucleotide binding assay.

5. A method according to claim 4 wherein the guanine nucleotide, of which the binding is determined, is Gpp (NH) p.

6. A method according to claim 4 or claim 5 wherein the guanine nucleotide is labeled.

7. A method according to any one of claims 1 to 6, for diagnosing psychiatric disorders according to claim 7 wherein said disorder is selected from the group comprising:

(I) mania
(ii) depression
(iii) panic, and
(iiii) schizophrenia

8. A method according to any one of claims 1-7 wherein the function of said at least one receptor-coupled G-protein is measured as an increase in agonist-induced guanine nucleotide binding capacity.

9. A method according to claim 8 wherein a value ≥ 45% increase in agonist-induced Gpp(NH)p binding capacity indicate hyperfunction, a value ≤ 15% increase in agonist-induced Gpp(NH)p binding capacity indicate hypofunction, and a value higher than 15% and lower than 45% increase in agonist-induced Gpp(NH)p binding capacity indicate normal function.

10. A method according to claim 7-9 wherein the function of at least two receptor-coupled G-proteins are determined and used in the diagnosis.

11. A method according to claim 10 wherein said receptor-coupled G-proteins include ß-adrenergic receptor-coupled G-protein and muscarinic receptor-coupled G-protein.

12. A method according to claim 11 wherein when the results of said determination indicate hyperfunction of ß-adrenergic receptor-coupled G- protein, and hyperfunction of muscarinic receptor-coupled G-protein, the psychiatric disorder is diagnosed as mania.

13. A method according to claim 11 wherein when the results of said determination indicate hypofunction of ß-adrenergic receptor-coupled G-protein, and hypofunction of muscarinic receptor-coupled G-protein, the psychiatric disorder is diagnosed as depression.

14. A method according to claim 11 wherein when the results of said determination indicate hyperfunction of β-adrenergic receptor-coupled G-protein, and hypofunction or normal function of muscarinic receptor-coupled G-protein, the psychiatric disorder is diagnosed as panic disorder.

15. A method according to claim 7-9 wherein the function of three receptor-coupled G-proteins are determined and used in the diagnosis.

16. A method according to claim 15 wherein said receptor-coupled G-proteins include ß-adrenergic receptor-coupled G-protein, muscarinic receptor-coupled G-protein and dopamine receptor-coupled G-protein.

17. A method according to claim 16 when the results of said determination indicate hyperfunction of dopamine receptor-coupled G-protein, normal

function of ß-adrenergic receptor-coupled G-protein and normal function of muscarinic receptor-coupled G-protein, the disorder is diagnosed as schizophrenia.

18. A method according to any one of claims 1-6 for gauging the effect of a treatment upon a patient with psychiatric disorder comprising:

(a) determining the function of at least one receptor-coupled G-protein, before and during the treatment;
(b) comparing said function of said at least one receptor-coupled G-protein, to that expected of a healthy person.

19. A method according to claim 18 wherein said psychiatric disorder is mania, and wherein decrease in β-adrenergic coupled G-protein function and/or muscarinic coupled G-protein function towards normal values indicate positive effect of a treatment.

20. A method according to claim 18 wherein said psychiatric disorder is depression and wherein increase in β-adrenergic coupled G-protein function and/or muscarinic coupled G-protein function toward normal values indicate positive effect of a treatment.

21. A method according to claim 18 wherein said psychiatric disorder is schizophrenia and wherein said decrease in dopamine-coupled G-protein function toward normal values indicate positive effect of a treatment.

22. A method according to claim 18 wherein said psychiatric disorder is panic disorder, and wherein decrease in β-adrenergic coupled G-protein function and/or increase in muscarinic coupled G-protein function towards normal values indicate positive effect of a treatment.

23. A method according to claim 1 or claim 2 for diagnosing psychiatric disorders or gauging the effect of a treatment upon a psychiatric patient wherein said determination is of $G\alpha_s$ and/or $G\alpha_i$ level.

24. A method according to claim 23 wherein said determination of $G\alpha_s$ and/or $G\alpha_i$ level is by a specific monoclonal or polyclonal antibody directed to said subunit.

25. A method according to claim 23 and 25 for diagnosing psychiatric dis-orders wherein said disorders are selected from the group comprising:

(I) mania
(ii) depression

26. A method according to claim 25 wherein when the results of said determination indicate over 15% increase in the level of $G\alpha_i$ and/or over 15% increase in the level of $G\alpha_s$, over the mean of control group the psychiatric disorder is diagnosed as mania.

27. A method according to claim 25 wherein when the results of said determination indicate decrease of >15% in the level of $G\alpha_i$ and/or in the level of $G\alpha_s$, relative to the mean of control group the psychiatric disorder is diagnosed as depression.

28. A method according to claim 23 or claim 24 for gauging the effect of a treatment upon a patient with psychiatric disorder wherein said psychiatric disorder is mania and wherein decrease in $G\alpha_s$ and/or $G\alpha_i$ indicate positive effect of the treatment.

29. A method according to claim 23 or claim 24 for gauging the effect of a treatment upon a patient with psychiatric disorder wherein said psychiatric disorder is depression and wherein increase in $G\alpha_S$ and/or $G\alpha_i$ indicate positive effect of the treatment.

30. A kit for diagnosing psychiatric disorders by monitoring the function of at least one receptor-coupled G-protein in MNL isolated from a patient, according to any one of claims 1 to 22, said kit comprising:

(a) agonists of one, two or three receptors, wherein said receptors are selected from the group comprising:
(I) ß-adrenergic receptor
(ii) muscarinic receptor
(iii) dopamine receptor

(b) labeled guanine nucleotide
(c) the manufacturer's instructions for using the kit

31. A kit according to claim 30 wherein the ß-adrenergic receptor agonist is isoproterenol; muscarinic receptor agonist is carbamylcholine; and dopaminergic receptor agonist is dopamine.

32. A kit for diagnosing psychiatric disorder by monitoring the amount of at least one type of Gα-subunit in the MNL isolated from a patient, according to any one of claims 1, 2 and 24 to 29, said kit comprising:

(a) 1 or 2 monoclonal or polyclonal antibodies selected from the group comprising anti-$G\alpha_S$ and anti-$G\alpha_i$;
(b) detectable probe which is capable of specifically binding to the antibody of (a);

(c) standard samples;

(d) the manufacturer's instructions for using the kit.

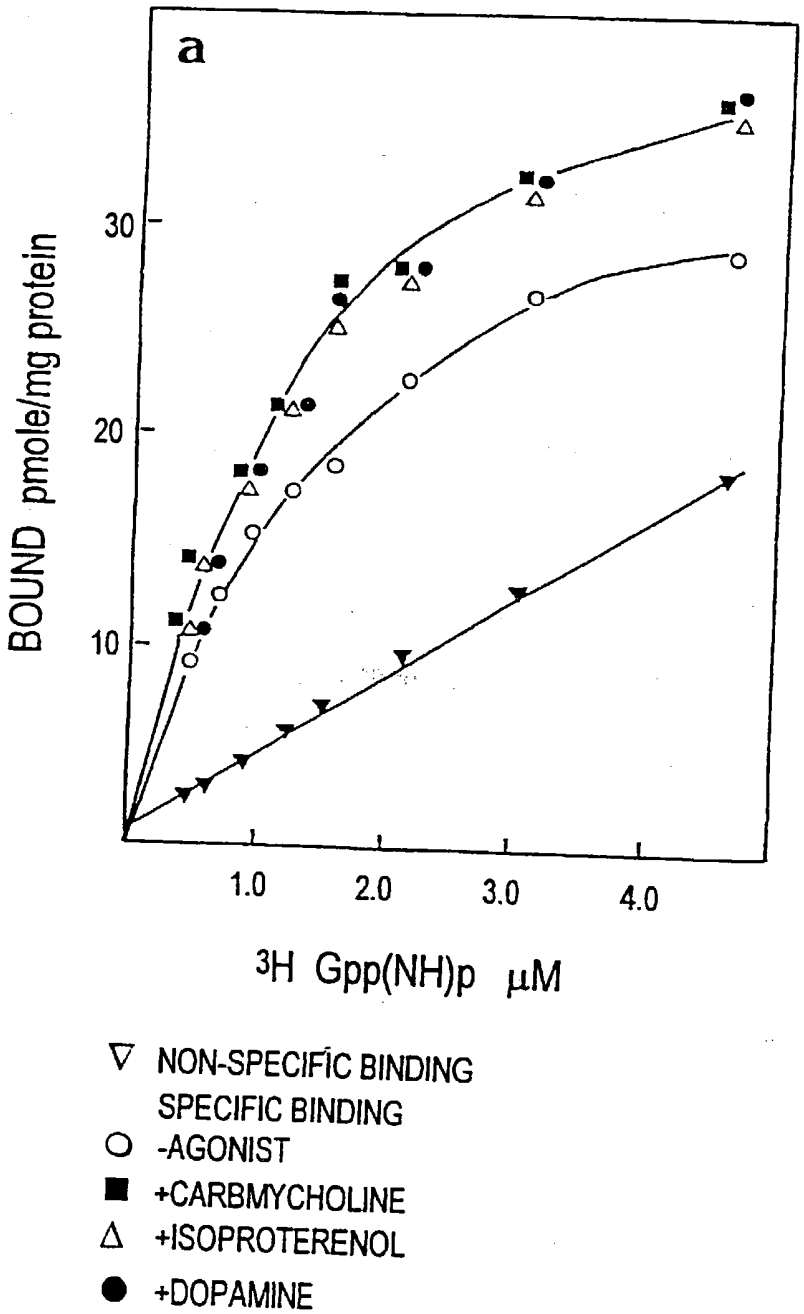

a
BOUND pmole/mg protein
30
20
10
1.0
2.0
3.0
4.0
3H Gpp(NH)p μM
NON-SPECIFIC BINDING
SPECIFIC BINDING
-AGONIST
+CARBMYCHOLINE
+ISOPROTERENOL
+DOPAMINE

Fig. 1

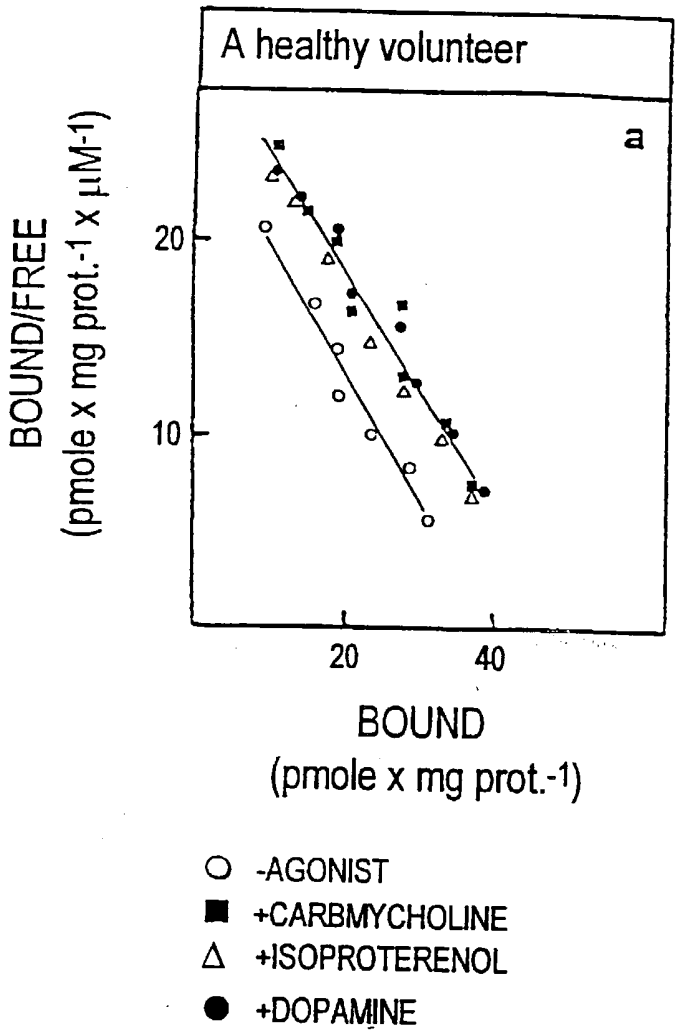

A healthy volunteer
a
BOUND/FREE
(pmole x mg prot.-1 x µM-1)
20
10
20
40
BOUND
(pmole x mg prot.-1)
-AGONIST
+CARBMYCHOLINE
+ISOPROTERENOL
+DOPAMINE

Fig. 2a

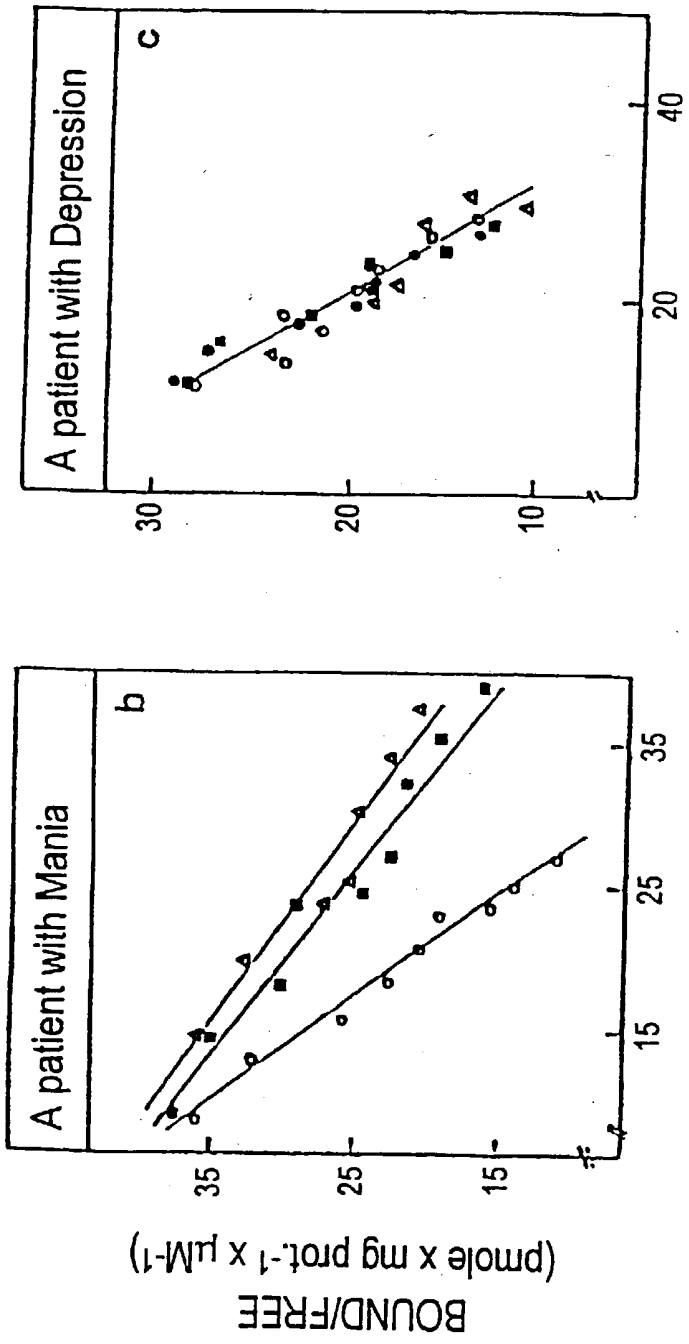

A patient with Mania
b
BOUND/FREE
(pmole x mg prot.-1 x μM-1)
35
25
15
BOUND
(pmole x mg prot.-1)
35
25
15
A patient with Depression
c
30
20
10
40
20
BOUND
(pmole x mg prot.-1)
○ -AGONIST
■ +CARBMYCHOLINE
△ +ISOPROTERENOL
● +DOPAMINE

Fig. 2b

Fig. 2c

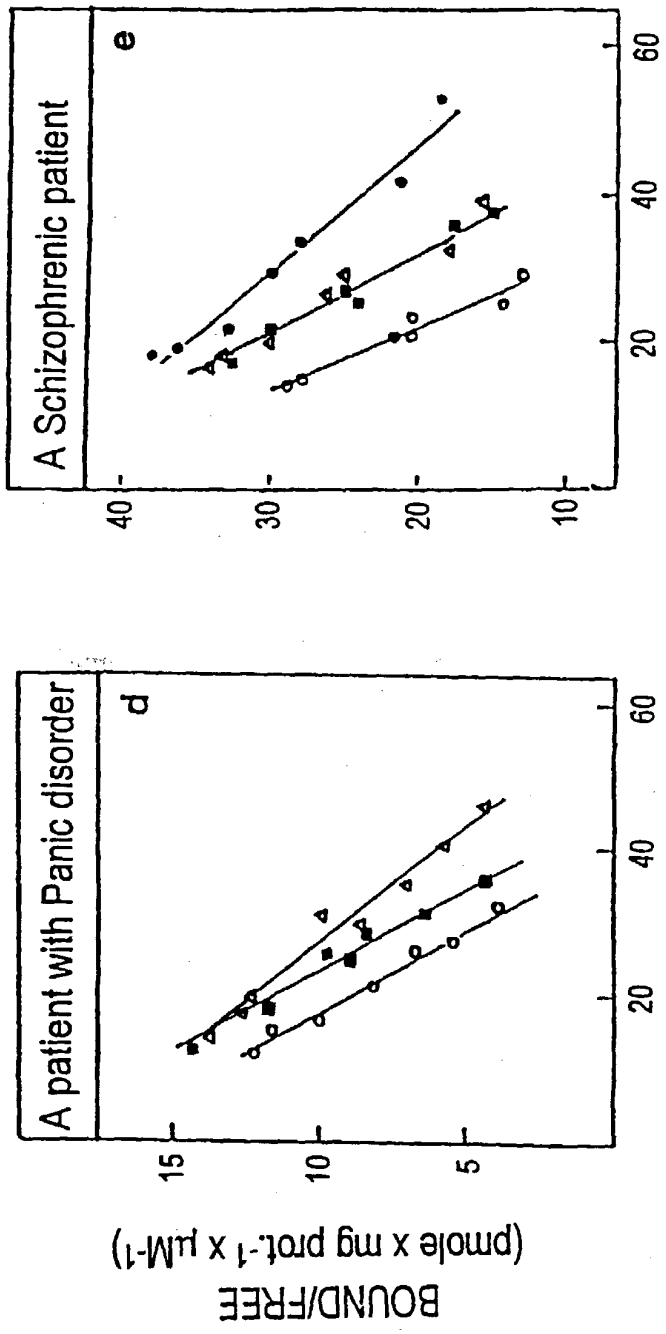

A patient with Panic disorder
d
A Schizophrenic patient
e
BOUND/FREE
(pmole x mg prot.-1 x μM-1)
BOUND
(pmole x mg prot.-1)
BOUND
(pmole x mg prot.-1)
O -AGONIST
■ +CARBMYCHOLINE
△ +ISOPROTERENOL
● +DOPAMINE

Fig. 2d

Fig. 2e

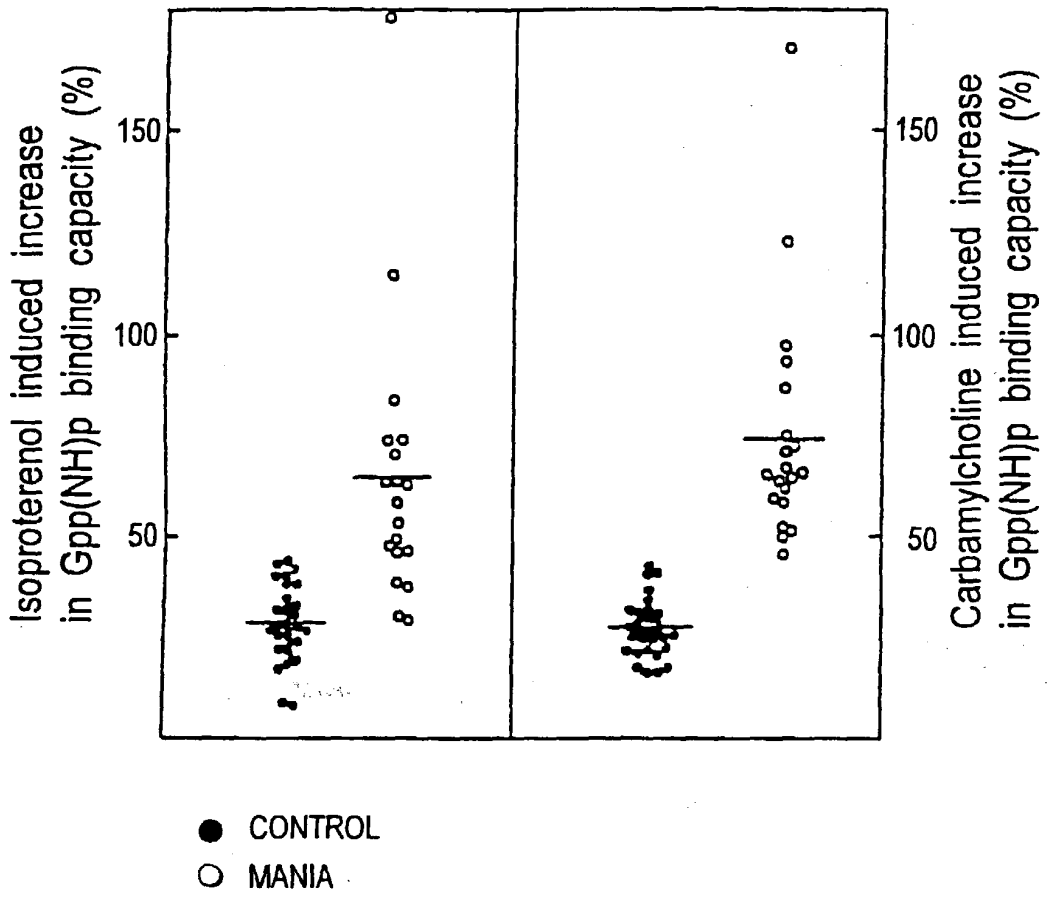
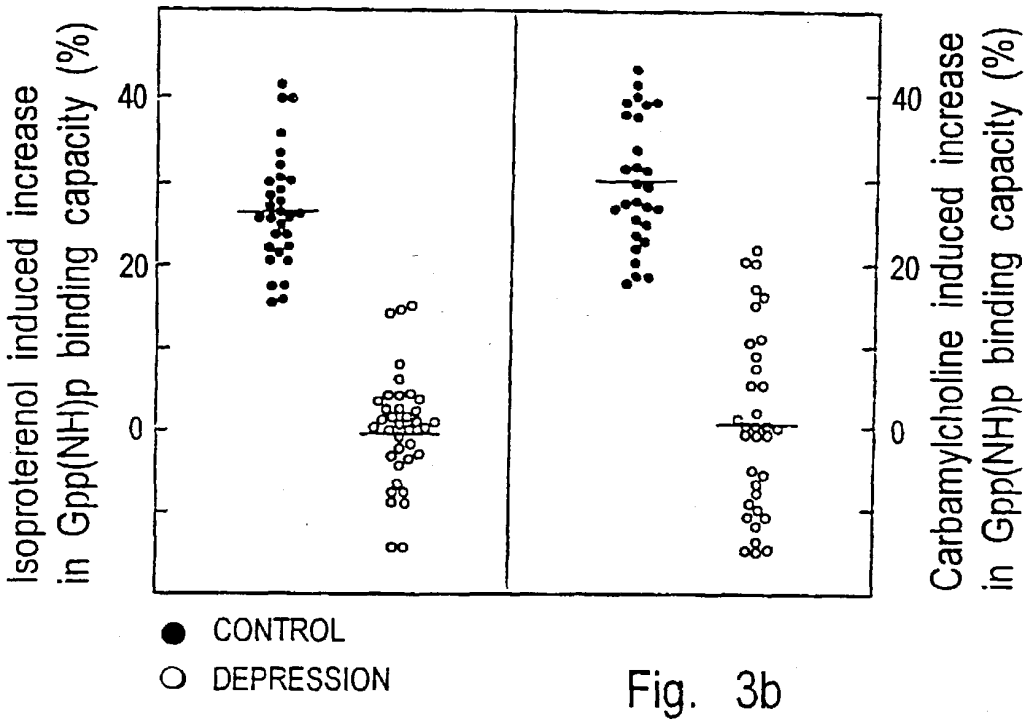

Isoproterenol induced increase in Gpp(NH)p binding capacity (%)
150
100
50
Carbamylcholine induced increase in Gpp(NH)p binding capacity (%)
150
100
50
CONTROL
MANIA

Fig. 3a


Isoproterenol induced increase in Gpp(NH)p binding capacity (%)
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20
0
Carbamylcholine induced increase in Gpp(NH)p binding capacity (%)
40
20
0
CONTROL
DEPRESSION

Fig. 3b

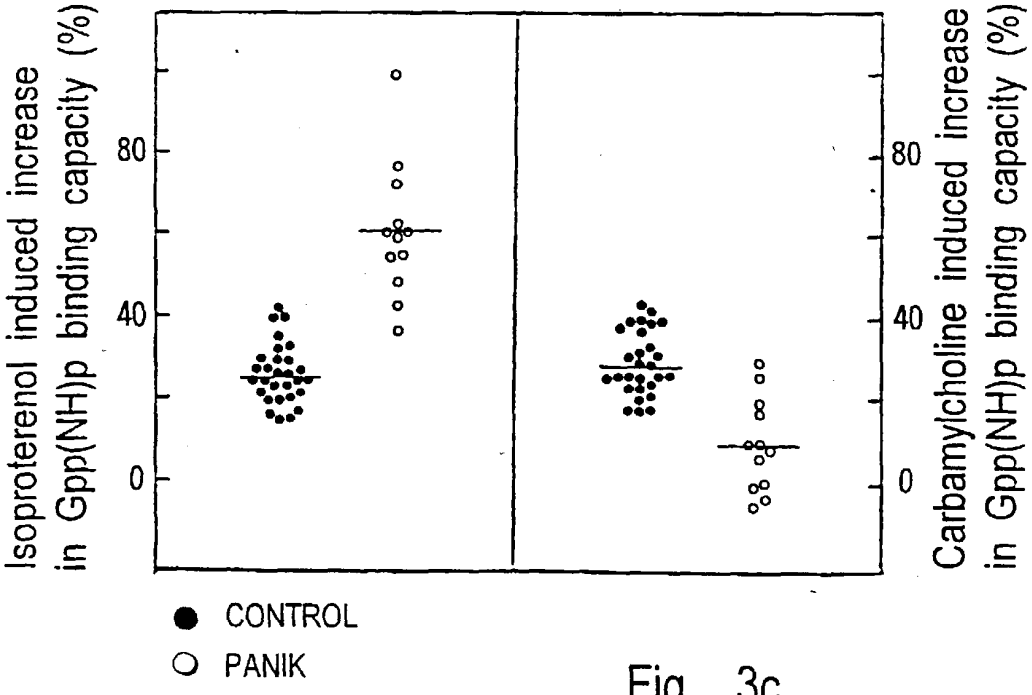

Isoproterenol induced increase in Gpp(NH)p binding capacity (%)
80
40
0
Carbamylcholine induced increase in Gpp(NH)p binding capacity (%)
80
40
0
CONTROL
PANIK

Fig. 3c

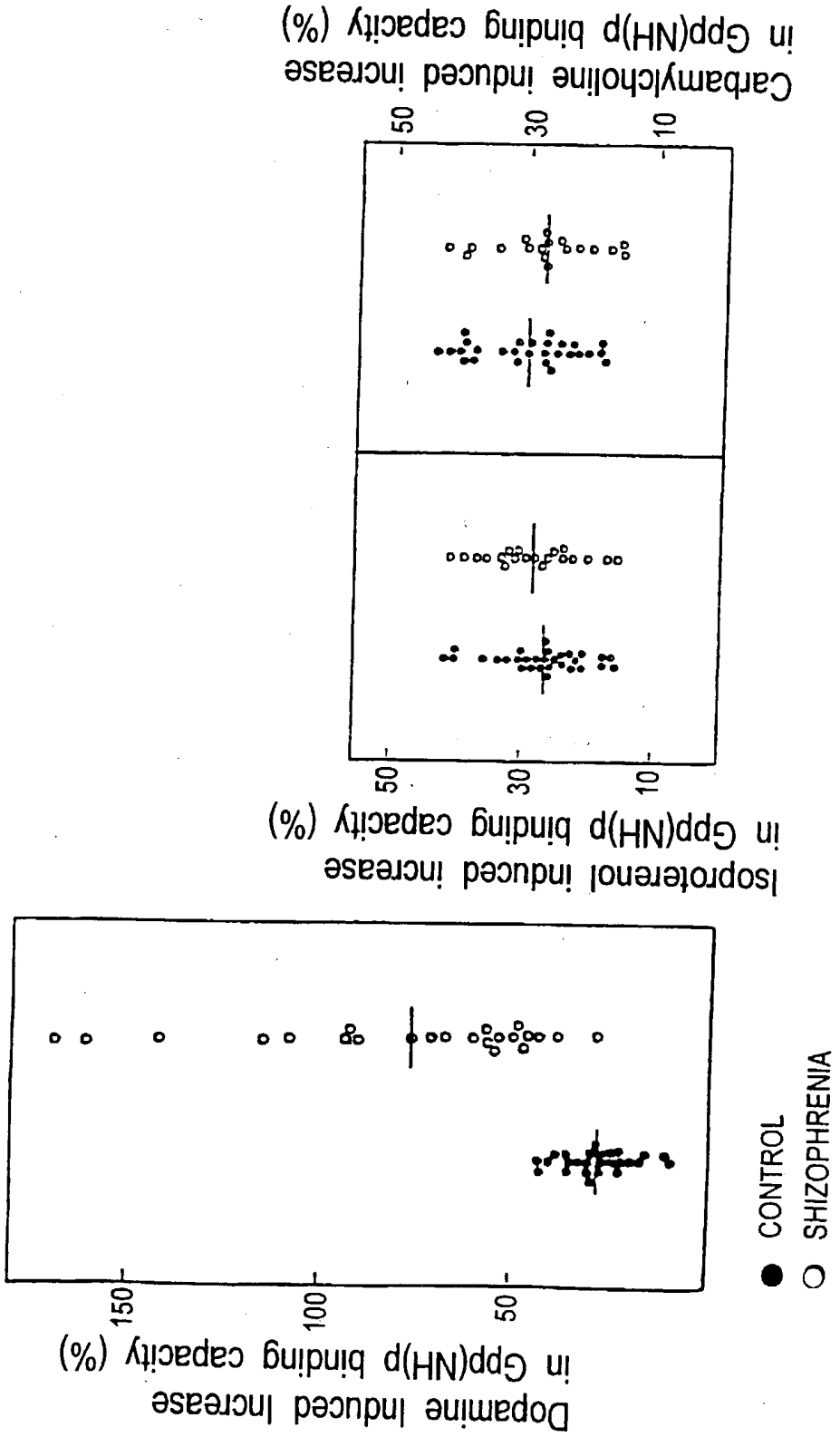

Carbamylcholine induced increase
in Gpp(NH)p binding capacity (%)
50
30
10
Isoproterenol induced increase
in Gpp(NH)p binding capacity (%)
50
30
10
Dopamine induced increase
in Gpp(NH)p binding capacity (%)
150
100
50
CONTROL
SHIZOPHRENIA

Fig. 3d

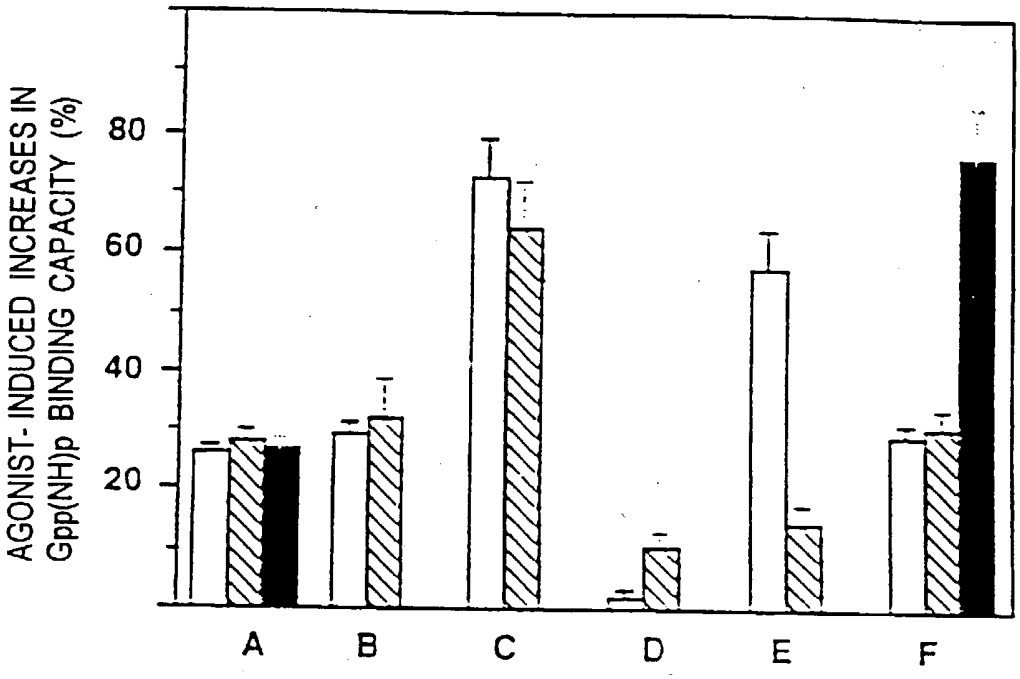

AGONIST- INDUCED INCREASES IN Gpp(NH)p BINDING CAPACITY (%)
80
60
40
20
A
B
C
D
E
F

Fig. 4

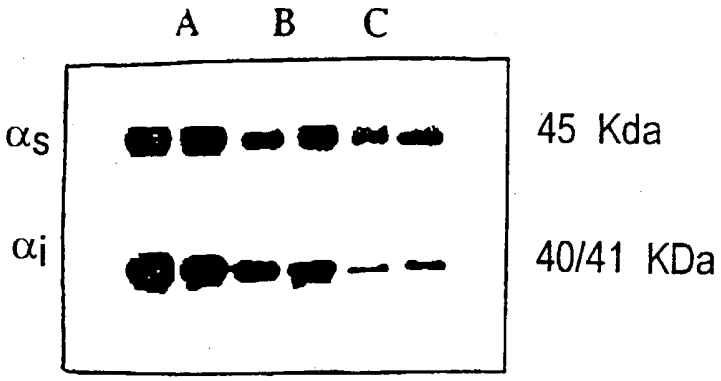

A
B
C
αs
αi
45 Kda
40/41 KDa

Fig. 5

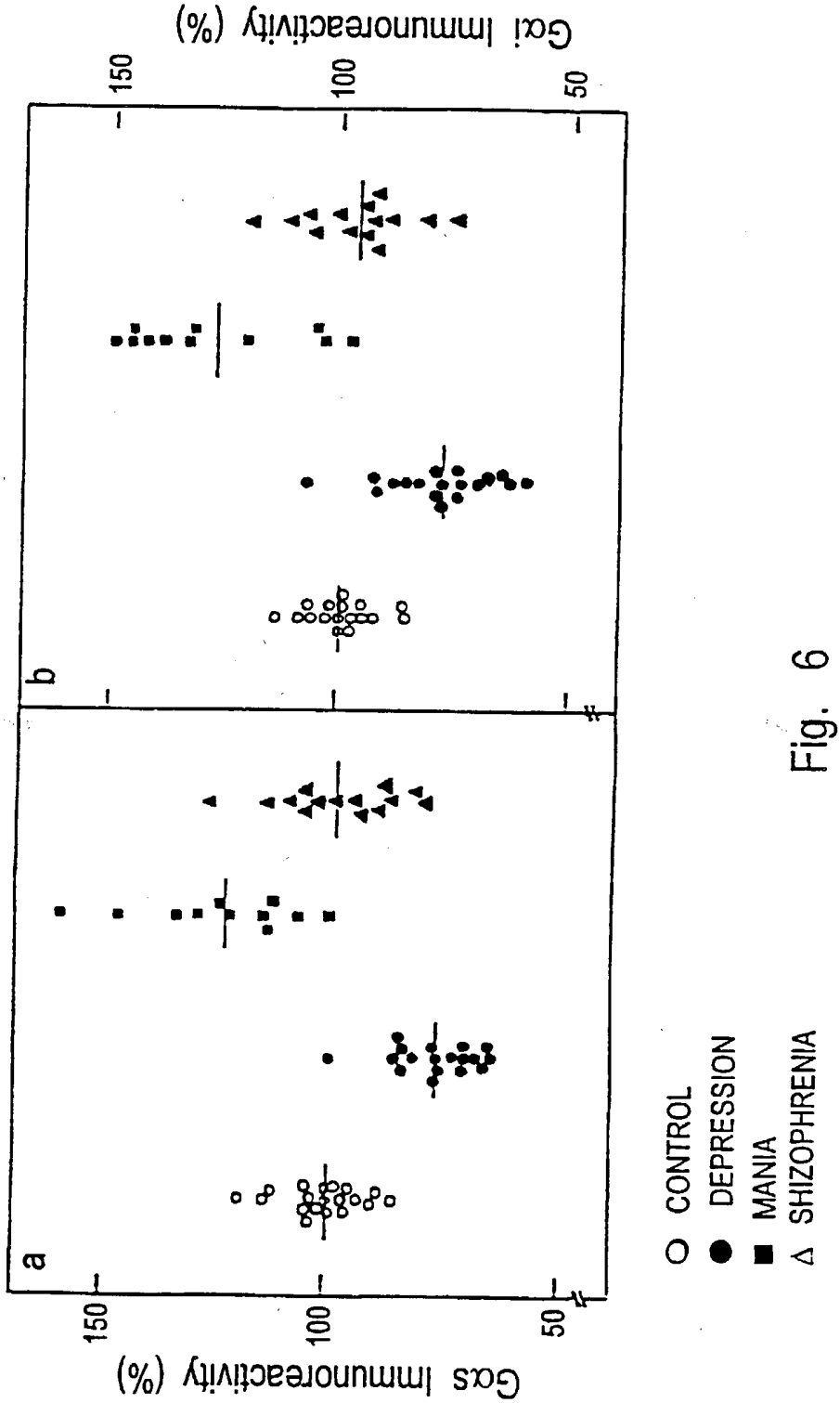

Gαs Immunoreactivity (%)
150
100
50
a
b
Gαi Immunoreactivity (%)
150
100
50
○ CONTROL
● DEPRESSION
■ MANIA
△ SHIZOPHRENIA

Fig. 6

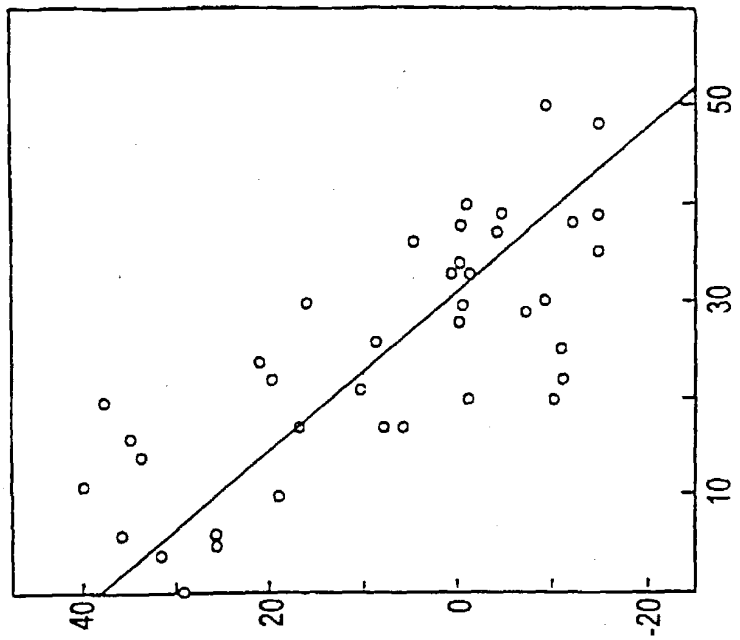

Carbamycholine Induced Increases
In Gpp(NH)p Binding Capacity (%)
40
20
0
-20
10
30
50
Beck Score

Fig. 7b

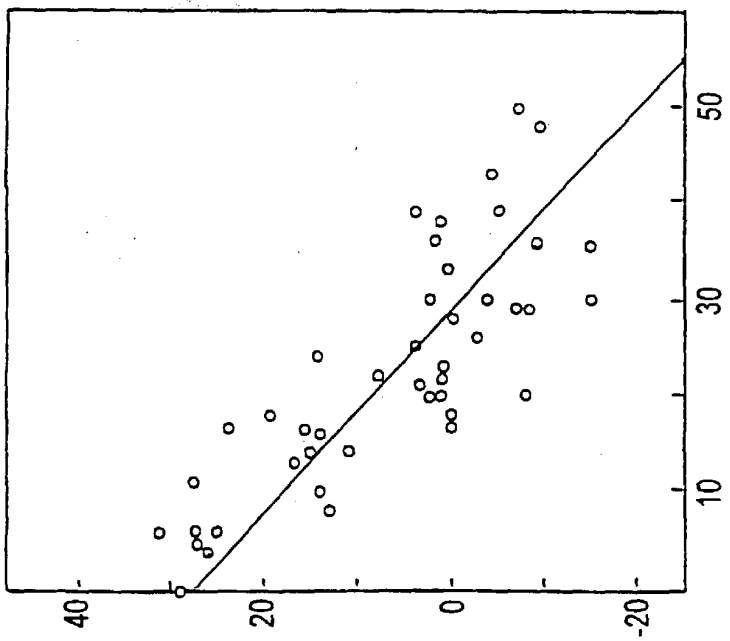
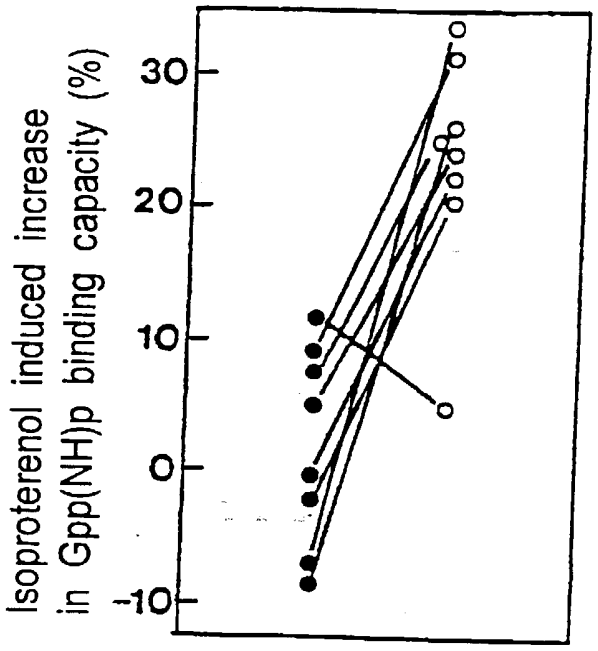

Isoproterenol Induced Increases
In Gpp(NH)p Binding Capacity (%)
40
20
0
-20
10
30
50
Beck Score

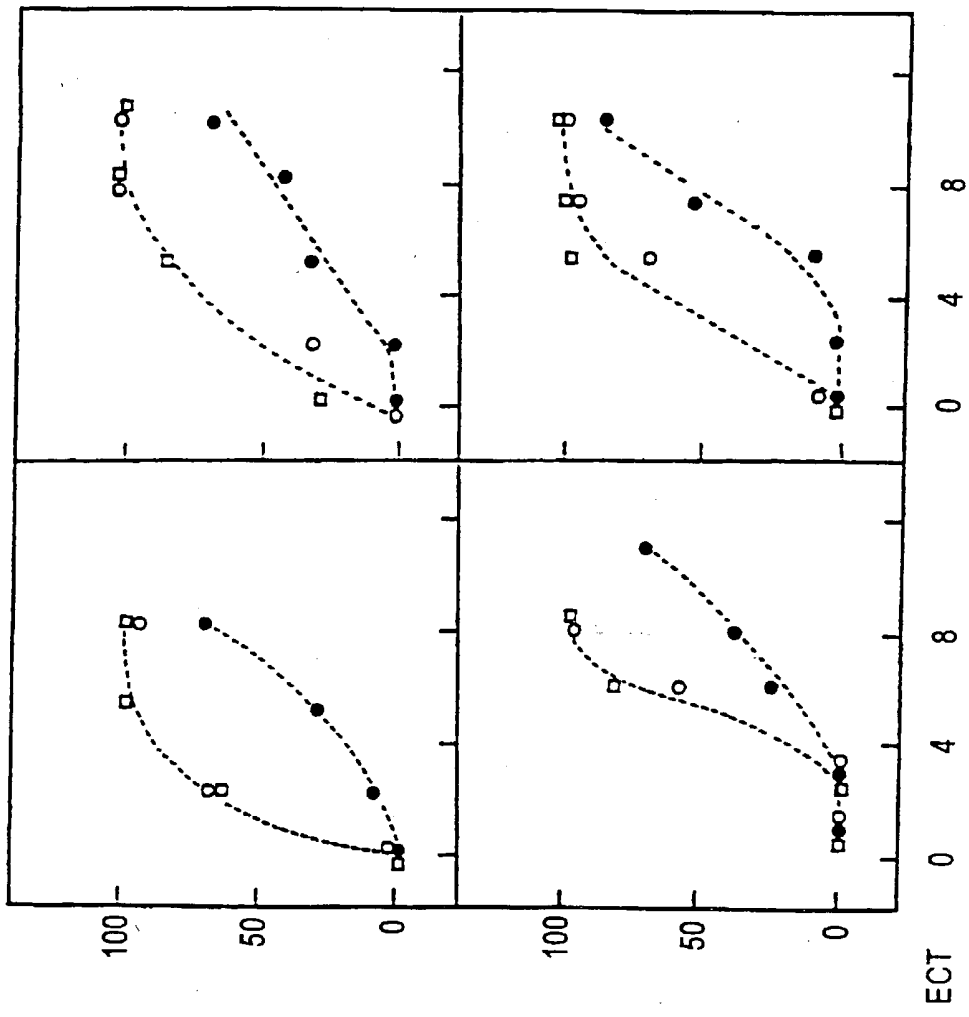
Fig. 7a



● before treatment

○ after treatment with antidepressants

Fig. 8



% Improvement In Beck Scoring (●),
β-G (○), And M-G (■) Function

Fig. 9a

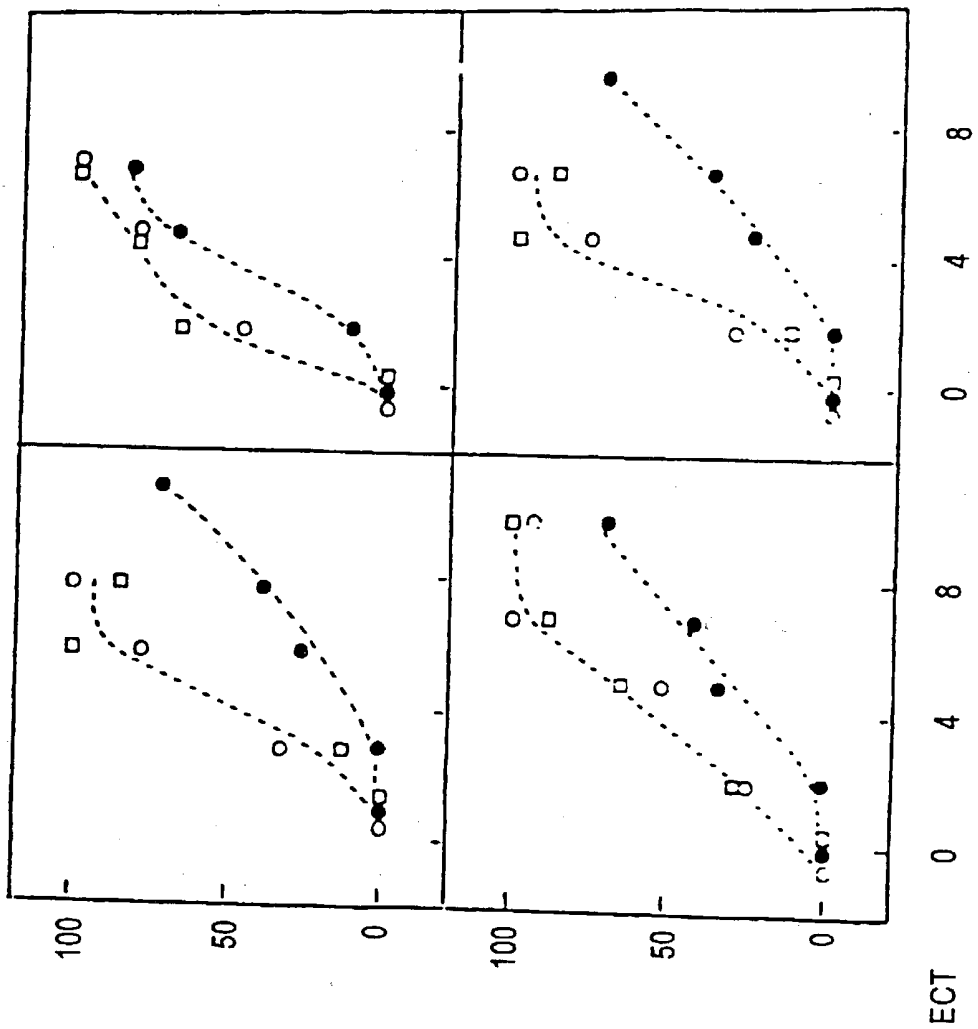

% Improvement In Beck Scoring (●),
Gαs (○), And Gαi (□) Immunoreactivity
100
50
0
100
50
0
0
4
8
0
4
8
ECT

Fig. 9b

INTERNATIONAL SEARCH REPORT

Inter nal Application No
PCT/IL 96/00166

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 G01N33/50 G01N33/566 G01N33/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	BIOLOGICAL PSYCHIATRY, vol. 29, no. 3, 1 February 1991, pages 273-280, XP000197092 SCHREIBER ET AL.: "Hyperfunctional G proteins in mononuclear leukocytes of patients with mania" cited in the application see the whole document ---	1-13,18, 19,30,31
A	BIOLOGICAL PSYCHIATRY, vol. 31, no. 5, 1 March 1992, pages 435-459, XP000197089 AVISSAR ET AL.: "The involvement of guanine nucleotide binding proteins .... " cited in the application --- -/--	

[X] Further documents are listed in the continuation of box C. [ ] Patent family members are listed in annex.

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

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Date of the actual completion of the international search	Date of mailing of the international search report
18 March 1997	0 4 -04- 1997
Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl, Fax (+ 31-70) 340-3016	Authorized officer Ceder, O

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Inte onal Application No
PCT/IL 96/00166

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,A	EXPERIMENTAL GERONTOLOGY, vol. 31, no. 3, 1996, pages 351-363, XP000197090 BARKI-HARRINGTON ET AL.: "Functional and quantitative measures of receptor-coupled ...." cited in the application see abstract -----	23,24

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